

**WE CLAIM:**

1. A method of producing hybrid seed by crossing a genetically transformed sterile parent plant with a suitable male fertile male parent plant, said genetically transformed fertile parent plant containing one or more recombinant DNA sequences comprising a sense or antisense gene, or both, which when expressed, blocks the production of tissues critical to pollen formation or function or renders said tissues susceptible to a chemical agent or physiological stress that blocks function of said tissues, said suitable male fertile male parent plant, when required to serve as a female restorer plant, containing a recombinant DNA sequence which compensates for the gene function that has been compromised or negates the disruption caused to tissues critical to pollen formation or function in said genetically transformed female parent plant.

2. A method of producing a hybrid seed with restored male fertility from plants selected from those species of pollen producing plants which are capable of being genetically transformed comprising the steps of:

- (a) producing a plant with a male sterile trait by a method comprising the steps of:
  - (1) transforming a plant cell of said plant with a sense gene which confers on said plant resistance to a chemical agent or a naturally occurring or artificially induced physiological or chemical stress;
  - (2) regenerating from said transformed plant cell a genetically transformed plant which is resistant to the same stress;

(3) inserting into the genome of a plant cell of the stress resistant plant a recombinant DNA molecule comprising:

- (i) a DNA sequence that codes for RNA that is complementary to the RNA sequence encoded by the said sense gene;
- (ii) a pollen specific promoter which functions in said plant cell to cause transcription of said DNA sequence into RNA; and
- (iii) a terminator sequence which defines a termination signal during transcription of said DNA sequence;

(4) obtaining a plant cell of said stress resistant plant which has been transformed with the gene described in step (c) above; and

(5) regenerating from said transformed plant cell a plant which has been genetically transformed with the genes described in step (a) and step (c) above and can be rendered male sterile by said chemical agent or stress; and

(b) increasing the number of genetically transformed male sterile plants by:

- (i) clonal propagation of said genetically transformed male sterile plant obtained in step (a) using tissue explants thereof, or other *in vitro* propagation techniques; or
- (ii) crossing said genetically transformed male sterile plant with a suitable male fertile plant;

- (iii) using said chemical agent or physiological stress to eliminate plants which are not genetically transformed with the DNA sequence amongst plants grown from seed produced by such cross; and
- (iv) repeating such cross over several generations with plants obtained in step (b)(iii) above in the presence of said chemical agent of physiological stress to increase the numbers of male sterile plants;
- (c) producing a male fertile restorer plant by:
  - (i) inserting into the genome of a plant cell of a suitable male parent plant that is capable of regeneration into a differentiated whole plant a gene that confers resistance to a chemical agent or a naturally occurring or artificially induced physiological stress, linked to a recombinant DNA sequence comprising:
    - A. a recombinant DNA molecule that comprises a modified form of said sense gene that does not contain the regions complementary to said antisense gene;
    - B. a promoter that functions in said plant to cause transcription of said modified DNA sequence at a time which restores the function of the sense gene, preferably at or about the time of the action of the anti-sense gene; and
    - C. a terminator sequence which defines a termination signal during transcription of said DNA sequence;
- (d) increasing the number of genetically transformed male fertile restorer plants in the fashion

described in (b) above or by selecting a plant homozygous for said restorer trait and increasing said plant by selfing in isolation; and

- (e) effecting a hybrid cross by pollinating said male sterile plants with pollen from said male fertile restorer plants.

3. A method to produce hybrid seed with restored male fertility from plants selected from those species of pollen producing plants which are capable of being genetically transformed comprising the steps of:

- (a) inserting into the genome of a plant cell of said pollen producing plant a gene which confers on said plant resistance to a chemical agent or physiological stress, and linked to said gene a recombinant DNA molecule comprising:
  - (i) a DNA sequence which codes for a cytotoxic molecule;
  - (ii) a pollen targeted promoter which functions in said plant cell to cause transcription of said DNA sequence; and
  - (iii) a terminator sequence which defines a termination signal during transcription of such DNA sequence;
- (b) obtaining a transformed plant cell;
- (c) regenerating from said plant cell a genetically transformed plant which is male sterile;
- (d) increasing the number of genetically transformed plants by:
  - (i) crossing the genetically transformed plant described in step (c) above with a suitable male fertile plant;

- (ii) using a chemical agent or physiological stress to eliminate plants which do not contain the genes described in step (a) above among plants grown from seed produced by such cross; and
- (iii) repeating such a cross over several generations with the plants obtained as in step (d)(ii) above in the presence of said chemical agent or physiological stress to increase the numbers of male sterile plants;
- (e) inserting into a plant cell of suitable male fertile plant selected from the same species a gene which confers on said plant resistance to a chemical agent or physiological stress and linked to said gene a recombinant DNA molecule comprising:
  - (i) a DNA sequence which codes for RNA that is complementary to the RNA sequence coding for said cytotoxic molecule;
  - (ii) a promoter which causes transcription of the DNA sequence defined in step (e)(i) above at or about the time of transcription of the DNA sequence defined in step (a)(i);
  - (iii) a terminator sequence which defines a termination signal during transcription of the DNA sequence described in step (e)(i) above;
- (f) obtaining a transformed plant cell from step (d);
- (g) regenerating from said transformed plant cell described in step (d) above a genetically transformed male fertile plant; and
- (h) producing a restorer line by:
  - (i) selfing the genetically transformed plant described in (g) and selecting from that selfing progeny, a plant homozygous for the male restorer trait;

- (ii) permitting self-fertilization of said plant homozygous for the male restorer trait; and
- (iii) growing seed of said plant, over a number of generations to increase the number of genetically transformed plants;
- (iv) effecting a hybrid cross by pollinating said male sterile plants with pollen from said genetically transformed male fertile plants.

4. A method of producing hybrid seed with restored male fertility from those species of pollen producing plants capable of being transformed and regenerated into a differentiated whole plant which method comprises:

- (a) (i) inserting into the genome of a plant cell of said plant that is capable of regeneration into a differentiated whole plant, a sense gene and linked to this a recombinant DNA molecule comprising:
  - A. a DNA sequence that when transcribed and translated codes for a cytotoxic molecule of a molecule which breaks down a substance into a cytotoxic molecule;
  - B. a pollen targeted promoter which functions in said plant to cause transcription of said DNA sequence into RNA at or about the time of the transcription of the sense gene in developing pollen; and
  - C. a terminator sequence which defines a termination signal during transcription of said DNA sequence:

- (ii) obtaining a transformed plant cell of said plant; and
- (iii) regenerating from said plant cell a plant which is genetically transformed with said DNA sequences described in (a)(i) above and is male sterile; and
- (b) increasing the number of genetically transformed male sterile plants by:
  - (i) clonal propagation of said genetically transformed male sterile plant described in step (a) using tissue explants thereof, or other in vitro propagation techniques; or
  - (ii) A. crossing the genetically transformed male sterile plant described in (a) with a isogeneic male fertile plant;  
B. using the chemical agent or physiological stress defined in (a)(ii) above to eliminate plants which do not contain the DNA sequence defined in (a)(ii) amongst plants grown from seed produced by such cross; and  
C. repeating such cross over several generations with plants obtained in step (b)(iii) above in the presence of said chemical agent or physiological stress to increase the numbers of male sterile plants;
- (c) producing a male fertile restorer plant by:
  - (i) inserting into the genome of a plant cell of a suitable male parent plant that is capable of regeneration into a differentiated whole plant a gene that confers resistance to a

chemical agent or a naturally occurring or artificially induced physiological stress, linked to a recombinant DNA sequence comprising:

- A. a gene that codes for a molecule that negates the disruption caused to tissues critical to pollen formation or function in said genetically transformed female parent plant;
  - B. a promoter that functions in said tissues critical to pollen formation or function to cause transcription of said gene into RNA at or about the time that the sense gene described in (a)(i) is active; and
  - C. a terminator sequence which defines a termination signal during transcription of said DNA sequence;
- (d) increasing the number of genetically transformed male fertile restorer plants by:
- (i) selfing the genetically transformed plant carrying the restorer trait described in (c), and selecting a plant homozygous for the restorer trait and increasing said plant by selfing in isolation; or
  - (ii) when applicable, conducting anther or isolated microspore culture of the genetically transformed plant carrying the restorer trait described in C. and selecting a plant homozygous for the restorer trait and increasing said plant by selfing in isolation.



- (e) effecting a hybrid cross by pollinating said male sterile plants described in (a) and increased in (b) in the presence of the chemical agent or physiological stress defined in (a)(ii), (if required), with pollen from male fertile restorer plants as described in (c) and increased in (d).

5. A method of producing hybrid seed from a plant selected from those species of pollen producing plants which are capable of being genetically transformed, which method comprises the steps of:

- (a) inserting into a genome of one or more plant cells of said plant the recombinant DNA molecule comprising:

- (1) one or more DNA sequences which may be the same or different, which encode a gene product which when produced in a cell of a plant which is essential to pollen formation and/or function is directly or indirectly capable of substantially interfering with the function and/or development of said cell; and

- (2) one or more promoters which may be the same or different, said promoters being capable of regulating the expression of said DNA sequences;

wherein the DNA sequences and promoters are selected such that the gene product selectively interferes with the function and/or development of a cell of a plant that is essential to pollen formation and/or function and whereby a plant regenerated from a cell of a plant having said recombinant DNA molecule integrated into its nuclear genome is substantially male-sterile or carries the male sterile trait; and

- (b) selecting a plant cell into which the recombinant DNA molecule is stably integrated;

- (c) regenerating from the selected plant cell a plant which carries the male sterile trait;
- (d) where appropriate, increasing the number of plants which carry the male sterile trait by selfing a plant which carries the male sterile trait, selecting a plant homozygous for the male sterile trait and increasing the homozygous plant by selfing in isolation, or by conducting anther or isolated microspore culture of a plant which carries the male sterile trait to select a plant which is homozygous for the trait and increasing said homozygous plant by selfing in isolation;
- (e) exposing said plant or plants to a sterility actuating agent which renders said plant or plants male sterile; and
- (f) crossing said male sterile plant or plants so obtained with a male fertile plant without said recombinant DNA molecule to obtain hybrid seed, said male fertile plant is selected such that the hybrid seed is capable of growing into a male fertile plant, said male fertile plant has a restorer DNA molecule encoding a restorer gene product integrated into its genome, said restorer DNA molecule or said restorer gene product being capable of restoring the function and/or development of a cell/tissue of a plant that is essential to pollen formation and/or function that is selectively interfered with by said gene product encoded by said DNA sequence of said recombinant DNA molecule.

6. A method of producing hybrid seed from a plant selected from those species of pollen producing plants which are capable of being genetically transformed which method comprises the steps of:

(a) inserting into the genome of one or more plant cells of said plant a recombinant DNA molecule comprising:

(1) one or more DNA sequences which may be the same or different, which encode a gene product which when produced in a cell of a plant which is essential to pollen formation and/or function is directly or indirectly capable of substantially interfering with the function and/or development of said cell; and

(2) one or more promoters which may be the same or different, said promoters being capable of regulating the expression of said DNA sequences;

wherein the DNA sequences and promoters are selected such that the gene product selectively interferes with the function and/or development of a cell of a plant that is essential to pollen formation and/or function and whereby a plant regenerated from a cell of a plant having said recombinant DNA molecule integrated into its nuclear genome is substantially male-sterile or carries the male sterile trait; and

(b) selecting a plant cell into which the recombinant DNA molecule is stably integrated;

(c) regenerating from the selected plant cell a plant which is male sterile;

(d) increasing the number of male sterile plants to produce a male sterile line by clonal propagation or by crossing the male sterile plant with a suitable male fertile plant and selecting plants which express said recombinant DNA molecules and repeating the steps of crossing and selecting as

required using progeny of such cross(es) which have been selected; and

- (e) crossing said male sterile line with a suitable male fertile plant line without said recombinant DNA molecule to obtain hybrid seed, said male fertile plant line is selected such that the hybrid seed is capable of growing into a male fertile plant, most plants of said male fertile plant line have at least one restorer DNA molecule encoding a restorer gene product integrated into their genome, said restorer DNA molecule or said restorer gene product being capable of restoring the function and/or development of a cell/tissue of a plant that is essential to pollen formation and/or function that is selectively interfered with by said gene recombinant DNA molecule.

7. Hybrid seed of a pollen producing plant having a nuclear genome incorporating one or more recombinant DNA molecules comprising:

- (a) one or more DNA sequences which may be the same or different, which encode a gene product which when produced in a cell of a plant which is essential to pollen formation and/or function is directly or indirectly capable of substantially interfering with the function and/or development of said cell; and

- (b) one or more promoters which may be the same or different, said promoters being capable of regulating the expression of said DNA sequences; and

wherein the DNA sequences and promoters are selected such that the gene product selectively interferes with the function and/or development of a cell of a plant that is essential to pollen

formation and/or function and whereby a plant regenerated from a cell of a plant having said recombinant DNA molecule integrated into its nuclear genome is substantially male-sterile or carries the male sterile trait and at least one restorer DNA molecule encoding a restorer gene product, said restorer DNA molecule or said restorer gene product being capable of restoring the function and/or development of a cell/tissue of a plant that is critical to pollen formation and/or function that is selectively interfered with by said gene product encoded by said DNA sequences of said recombinant DNA molecules.

8. A method of producing seed of a male sterile plant comprising:

- (a) producing a male sterile plant line comprising
  - (i) introducing into the genome of one or more plant cells of a pollen-producing plant a first recombinant DNA molecule comprising:
    - (1) one or more DNA sequences which may be the same or different, which encode a gene product which when produced in a cell of a plant which is essential to pollen formation and/or function is directly or indirectly capable of substantially interfering with the function and/or development of said cell; and
    - (2) one or more promoters which may be the same or different, said promoters being capable of regulating the expression of said DNA sequences; and

wherein the DNA sequences and promoters are selected such that the gene product selectively interferes with the function and/or development of a cell of a plant that is essential to pollen formation and/or function and whereby a plant regenerated from a

cell of a plant having said recombinant DNA molecule integrated into its nuclear genome is substantially male-sterile or carries the male sterile trait

said first recombinant DNA molecule comprises a pollen specific promoter and a selection marker gene which encodes a selection gene product which permits the selection of a plant having said first recombinant DNA molecule integrated in its genome;

- (ii) selecting a plant cell into which the first recombinant DNA molecule is stably integrated;
  - (iii) regenerating from the selected plant cell a plant which carries the male sterile trait;
  - (iv) increasing the number of plants which carry the male sterile trait to produce a plant line having plants carrying the male sterile trait; and
  - (v) exposing said plant line to the non-toxic substance to render plants of said plant line male sterile;
- (b) cross pollinating plants of the male sterile plant line obtained in (a) with plants of a second plant line having a genome which stably incorporates a second recombinant DNA molecule comprising a second DNA sequence encoding a second gene product which is capable of converting a substance which is endogenous to cells of plants of said second plant line, to said non-toxic substance; a second promoter capable of regulating the expression of said second DNA sequence; said first and second recombinant DNA molecules are incorporated into homologous chromosome pairs, and wherein plants of said second plant line are not capable of

rendering the non-toxic substance cytotoxic to cells of plants of said second line which are essential to pollen formation and/or function; and

- (c) harvesting seed of plants of said male sterile plant line.

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